CHAPTER II

LITERATURE REVIEW

1. Microencapsulation

Encapsulation is a process by which active substances are incorporated inside particles in order to protect them from external environment, which is generally to improve its performance, enhance its shelf life stability, and to control the release of active substances from the encapsulated particles. Particle size that are larger than 1,000 μm are known as macroparticles. Those below 1 μm, they are known as nanoparticles and those whose diameter between 1–1,000 μm are known as microparticles. Microencapsulation is an encapsulation process in which very small particles, microspheres of matrix particles or droplets of liquid materials are surrounded by coating to give small capsules. The product obtained by this process is called as microcapsules or microspheres as shown in Figure 1 which have difference in morphology and microstructure [3, 17, 18].

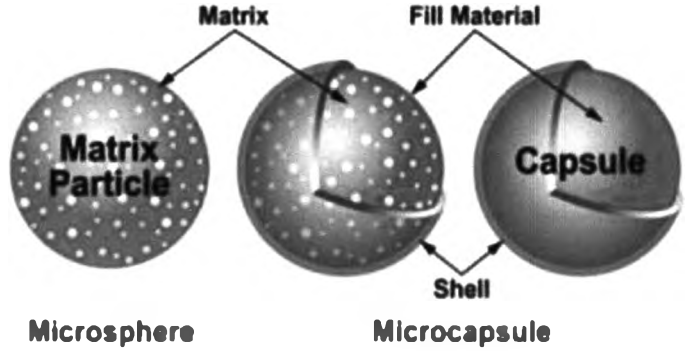


Figure 1 Characteristics of microsphere and microcapsule

Microencapsulated particles consist of two main components namely core material and coat or shell material. Core material usually contains the active substance, while coat or shell material covers or protects the core material. Many types of active substances such as active pharmaceutical ingredients, proteins, peptides, volatile oils, food ingredients, pigments, dyes, monomers, catalysts and pesticides can be encapsulated with different types of shell materials. For specific encapsulation reasons, various types of natural polymers, synthetic and semi-synthetic polymers have been used as shell materials such as cellulose derivatives, starch, gelatin, chitosan, polyesters, polyanhydrides and polyphosphazenes. Most

designs of delivery systems using natural polymers have been based on proteins (e.g., collagen, gelatin, and albumin) and polysaccharides (e.g., starch, dextran, hyaluronic acid, pectin and chitosan). The microencapsulation technology has a wide range of industrial applications such as in pharmaceuticals, nutraceuticals, cosmetics, food, agrochemicals, industrial chemical, etc. In pharmaceutical applications, this technology has received much attention in the design of controlled release and targeted dosage forms with suitable biopolymers. It also includes the conversion of liquid active substance into dry solid particles, the separation of incompatible components for functional reasons, the masking undesired properties of the active substances such as color, taste, and odor, the avoidance of adverse effects like gastric irritation of the drug, and the need of a safety in the handling of toxic materials. [17, 18].

The technique of microencapsulation depends on the physical and chemical properties of the active substances to be encapsulated. There are many factors to consider for selecting the processes. The different techniques of microencapsulation can produce the different type of microparticles which defined as matrix type and core shell type (microcapsule) microparticles. Various encapsulation techniques are available and have broadly divided into two types of processes, one being a physical process, such as spray drying (SD), spray chilling, fluid bed coating, pan coating, air suspension coating, freeze drying (FD), rotary disk atomization, nozzle co-extrusion, centrifugal extrusion and the other one being a chemical process, such as coacervation phase separation, ionic gelation, solvent evaporation, solvent extraction, liposome technology, interfacial polymerization, matrix polymerization. Among these various encapsulation techniques, spray drying and freeze drying are the two processes notably used to support an industrial production and also to increase the stability of active substance [2, 3, 18].

1.1 Spray drying process

Spray drying serves as a microencapsulation technique in which an active material is dissolved or suspended in a melt or polymer solution and becomes trapped in the dried particle. The process involves a continuous transformation of a feed from a fluid state into a dried particulate form. A substance to be encapsulated and the coating materials are homogenized as a solution or suspension in water. The mixture is then feed into a spray dryer. The sprays are produces by a nozzle atomizer and contacting the small droplets with hot air in the chamber. The droplets moisture evaporation and cried particles formation produce under controlled temperature

and airflow conditions. Microparticles are discharged continuously from the drying chamber [2].

Microencapsulation by spray drying is an economical process which is mostly used in various industries. The process is very rapid and production capacity can be designed up to several hundred tons per hour. The main advantage of spray drying is a versatile and high performance technology, evident on the multiple applications and the wide range of products that can be obtained. Since this technology using a high drying air temperature with a rising up to a hundred degrees celsius of the inlet temperature in drying medium, but most of the time in the entire drying process the material temperature does not exceed the wet-bulb temperature of the air in the dryer due to water rapidly evaporates and cools down the droplets in a short contact time and it is a continuous process which particularly suitable to handle labile materials. However, spray drying requires huge costs for the equipments required and its continuous operation. The equipments are expensive, regardless of atomizer type. Spray dryers generally have low thermal efficiencies due to a large volume of hot air that circulates in the drying chamber without contacting to the particles [2, 18].

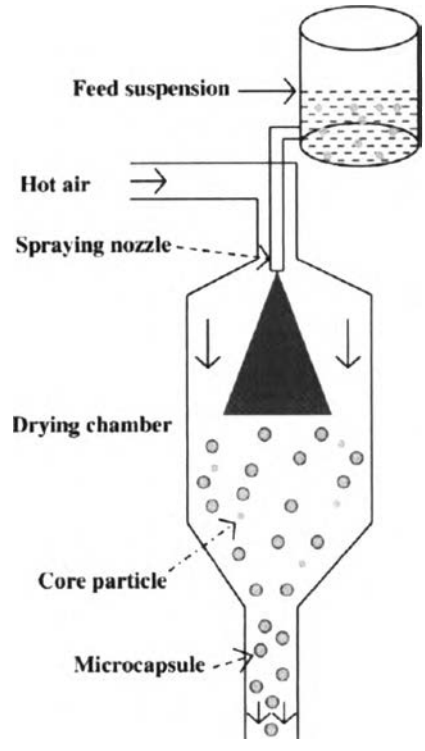


Figure 2 Composition and sample flow of spray drying technique

In general, the microparticles produced by spray drying are matrix type. The core exists as microparticles or microdroplets distributed within the dry solid matrix. The spray dryer is easy and convenience to operate. The end-product can be controlled and comply with precise quality standards regarding particle size distribution, residual moisture content, bulk density, and particle shape. An important pharmaceutical spray dried product properties such as a uniform of particle size, spherical shape and better solubility can be achieved. The process parameter factors during spray drying such as a drying process conditions; atomized droplet size, dryer inlet air and outlet air temperatures, drying air velocity, drying feed temperature, humidity of the dryer inlet air are being consider. In addition, a property of the volatile compound such as its concentration, molecular weight, vapor pressure, and a property of the capsule wall material such as type and molecular weight are also influenced in the process. Operating conditions and dryer design are selected and modified according to the drying characteristics of the product and particle specification [2, 19, 20].

1.2 Freeze drying process

Freeze drying, known as lyophilization, is a dehydration process typically used to preserve a material or make the material more suitable for transport. Freeze drying works by freezing the material then reducing the pressure and increase temperature to allow the ice forming in the material to sublimate directly from the solid phase to the gas phase [21, 22].

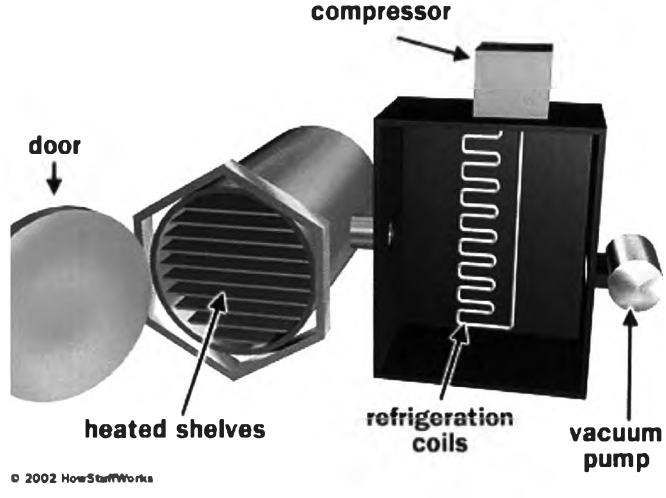


Figure 3 Composition of freeze drying machine

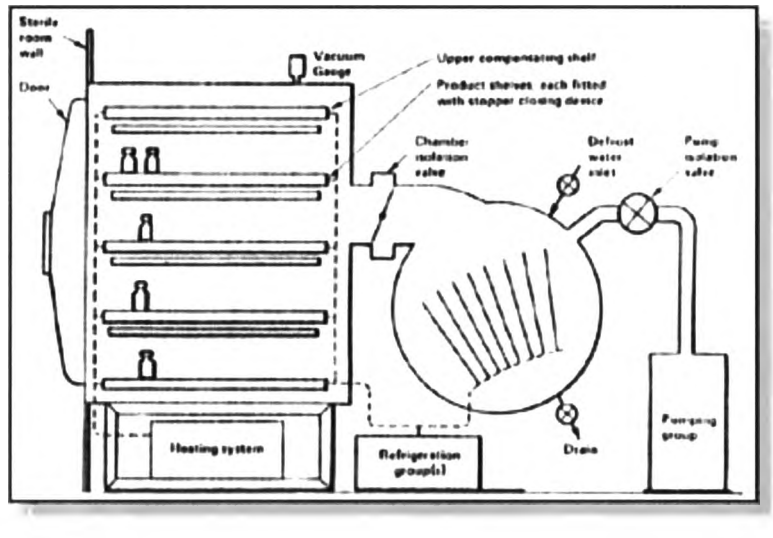


Figure 4 Composition of freeze drying machine

The three major stages of a freeze drying process are freezing, primary drying (sublimation), and secondary drying (desorption). Each stage is critical in producing of required end products. An optimization of controllable stages of freeze drying process is that which achieves the highest drug quality for the least cost [21].

Freezing, the first stage, freezing will ensure a good cake, while improper freezing may make a product result that cannot be successfully freeze dried. The objective of freezing is to proceed a frozen matrix with sufficient crystal structure in order to allow the sublimating material to sublimate. The larger the crystal structure, the better the sublimation. A slow freezing yields a larger crystal. The products form a glassy material and annealing may be required during the freezing process. Annealing, first set the lower temperature then increasing the temperature and then lowering it again, locks the constituents in place and then allows the crystals to grow. Freezing time can proceed from 1 hour to 24 hours, depending on the material and its application. Primary drying (sublimation), the second stage, performs for allowing the unbound moisture out of the product. Sublimation occurs under vacuum with the product temperature below its critical temperature. This step is usually the longest process. At the end of the primary drying step, the product will have approximately 3 to 5% of moisture content. Secondary drying (desorption), the third stage, drives an ionic bound water out from the materials which done by heating up the product. This secondary drying can lower a moisture levels to 0.5% [1, 21, 22].

Freeze-dried products can be rehydrated (reconstituted) much more quickly and easily because microscopic pores occurred by the process. The pores are created by the ice crystal that sublimate with leaving gaps or pores inside. This is particularly important for pharmaceutical application. Pharmaceutical industry often use freeze drying technique to improve the product shelf life, such as vaccines and other biological products. The products can be easily stored, shipped, and reconstituted to its original form for injection. Another example from the pharmaceutical and food industry is the use of freeze drying to produce tablets or wafers, the advantage of which is less excipient as well as a rapidly absorbed and easily administered dosage form [1, 23-25].

2. Plant tissue culture

Figure 5 Plant tissue culture

Plant tissue culture is a techniques for maintaining or growing plant cells, calluses, tissues and organs under sterile conditions on a culture medium. Plant tissue culture is widely used to produce some part of a plant or callus that similar to the explant in a method known as micropropagation. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation. Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency). Single cells, plant cells without cell walls, pieces of leaves, stems or roots can be used to produce a new plant or new cells on culture media which composed the required nutrients and required plant hormones. The most commonly used tissue explants are the meristematic ends of the plants like the stem tip, auxiliary bud tip and root tip. These tissues have high rates of cell division and either concentrate or produce required growth hormones including auxins and cytokinins [26-28].

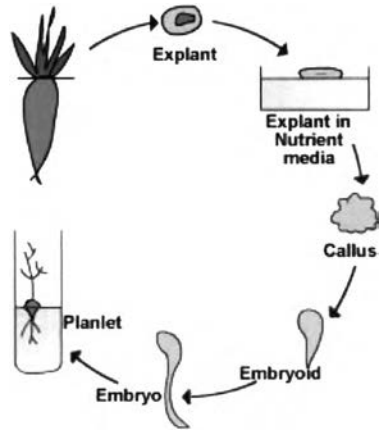


Figure 6 Cycle of tissue culture from explant to plant via callus induction

Plant tissue culture is a normally used procedure in plant biology in which organism is planted from the explants on a nutrient medium under sterile conditions. In addition, plants are a wide source of secondary metabolites, which can be used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, biopesticides and food additives. There are a number of plant cell cultures that producing a higher amount of secondary metabolites than in normal plants. However, there are still problems in the production of metabolites cell cultures resulting from the instability of cell lines, low yields, slow growth and scale-up problems [27, 29-31]. In addition, plant cell culture product provides the genetics and epigenetic variations. The epigenetic factors, which are produced in undifferentiated cell from cell culture can affect the growth and proliferation of the cell [12, 13] so this effect could be useful in wound healing. In summary of plant tissue part, the extract of plant callus can provide the secondary metabolite and the epigenetic factors to synergist the effect for accelerating wound closure.

Table 1 Products of plant cell culture from various plant species and use [27]

Product	Use	Plant species
Ajmalicine	Antihypertensive	*Cath. roseus*
Artemisinin	Antimalarial	*Artemisia annua*
Ajmaline	–	*Ra. serpentina*
Acinitine	–	*Acotinum* spp.
Berberine	Intestinal ailment	*C. japonica*
Camptothecin	Antitumour	*Camptotheca acuminata*
Capsaicin	Counterirritant	*Ca. frutescens*
Castanospermine	Glycoside inhibitor	*Castanospermum australe*
Codeine	Sedative	*P. somniferum*
Colchicine	Antitumour	*Colchium autumnale*
Digoxin	Heart stimulant	*Di. lanata*
Diosgenin	Steroidal precursor	*Dioscorea deltoidea*
Ellipticine	Antitumour	*Orchrosia elliptica*
Emetine	–	*Cephaclis ipecacuanha*
Forskolin	Bronchial asthma	*Coleus forskolii*
Ginsenosides	Health tonic	*Panax ginseng*
Morphine	Sedative	*P. somniferum*
Podophyllotoxin	Antitumour	*Podophyllum petalum*
Quinine	Antimalarial	*Cinchon. ledgeriana*
Sanguinarine	Antiplaque	*Sanguinaria canadensis*
		P. somniferum
Shikonin	Antibacterial	*L. erythrorhizon*
Taxol	Anticancer	*Taxus brevifolia*
Vincristine	Antileukemic	*Cath. roseus*
Vinblastine	Antileukemic	*Cath. roseus*

Table 2 Secondary metabolites extracted from various plant cell culture and yield [27]

High yields of secondary products

Product	Plant species	Yield (% D.W.)	Reference
Rosmarinic acid	*Sa. officinalis*	36.0	Hippolyte et al. (1992)
Rosmarinic acid	*Col. blumei*	21.4	Ulbrich et al. (1985)
Anthroquinones	*M. citrifolia*	18.0	Zenk et al. (1975)
Shikonin	*L. erythrorhizon*	12.4	Fujita (1988)
Berberine	*Th. minus*	10.6	Kobayashi et al. (1988)
Jatrorhizine	*Berberis wilsonae*	10.0	Breuling et al. (1985)
Anthocyanins	*Pe. frutescens*	8.9	Zhong et al. (1994)
Berberine	*C. japonica*	7.5	Matsubara et al. (1989)
Diosgenin	*Diosc. deltoidea*	3.8	Sahai and Knuth (1985)
Sanguinarine	*P. somniferum*	2.5	Park et al. (1992)
Serpentine	*Cath. roseus*	2.2	Zenk et al. (1977)

Adapted from Ravishankar and Ramachandra Rao (2000).

2.1 *Thunbergia laurifolia* Linn. callus

Rang chuet is the Thai vernacular name of medicinal plants. Among families, *T. laurifolia* is the best known and the most commonly used species. It is a woody climbing plant in the Acanthaceae family. This specie has widely used for its detoxifying effects against insecticides, ethanolic and metallic poisons and also for the treatment of drug addiction. Anti-inflammatory, anti-diabetic and antipyretic activities were also reported [14]. In 2012, Wonkchalee. O., reported that *T. laurifolia* possessed antioxidant and anti-inflammatory properties as well as anticancer activities and reducing inflammation from pathological changes in syrian hamsters infected with the human liver fluke *Opisthorchis viverrini*. The finding suggested that *T. laurifolia* possessed antioxidant and anti-inflammatory properties and that its application might be useful for hepatoprotective and prevention of the inflammatory process, which is one of the risk factors of *O. viverrini*-associated cholangiocarcinoma (CCA) [32].

Figure 7 *Thunbergia laurifolia* leaves

T. laurifolia leaves are opposite, heart-shaped with serrated leaf margin and taper to a pointed tip. Flowers are not scented and borne on pendulous inflorescences. The hermaphrodite flower is trumpet-shaped with a short broad tube, white outside and yellowish inside. The corolla is pale blue in colour with 5–7 petals, one larger than the others. In 2013, Suwanchaikasem P., et al. reported and compared an antioxidant activity and TLC, PCR-RFLP fingerprints from three species of Rang Chuet of *T. laurifolia, Crotalaria spectabilis* and *Curcuma* aff. *amada*. The results showed that *T. laurifolia* exhibited the highest free radical scavenging and

ferric reducing properties of the three aqueous extracts and the flavonoids from ethanolic extracts also the phenolic compounds from aqueous extract were observed [33]. They also reported the antioxidant-guided isolation of rosmarinic acid from *T. laurifolia* to use as a bioactive marker for standardization [15].

Figure 8 Callus induction and culture on hard media

Plant callus is a mass of unorganized parenchyma cells derived from plant tissue (explants) for use in biological research and biotechnology. In plant biology, callus cells are those cells that generate at a plant wound. Callus formation is induced from plant tissues after surface sterilization and plating onto the culture medium. Plant growth regulators, such as auxins, cytokinins, and gibberellins, are supplemented into the culture medium to induce the callus formation or somatic embryogenesis and plant callus is usually derived from somatic tissues. The plant tissues which initiated a callus formation depends on plant species and tissues that are available for explant culture. The cells that offer the rise to callus and somatic embryos usually undergo rapid division or are undifferentiated such as meristematic tissue. Callus can be produced from a single differentiated cell, and many callus cells are totipotent, being able to regenerate the whole plant body. Callus cultures are often classified as being either compact or friable. Friable calluses can be separate apart easily, and can be used to generate the cell suspension cultures. Plant callus culture is typically sustained on gel medium. Callus induction medium consists of agar and a mixture of macronutrients and micronutrients for the given cell type. Several types of basal salt mixtures such as a modified Murashige and Skoog

medium have been used in plant cell culture and was studied for a specific type of each plants [26, 28, 31].

Plant callus culture has been widely used in both basic research and industrial applications. Auxin and cytokinin have been widely used to generate callus. A balance between two plant hormones determines the state of differentiation and dedifferentiation. Exogenous application of auxin and cytokinin induces callus in various plant species. Other hormones, such as brassinosteroids or abscisic acid, also induce callus and may substitute of auxin or cytokinin for callus formation in some of plant species. Since the production of secondary metabolites is generally higher in differentiated tissues, there are attempts to cultivate shoot cultures and root cultures for the production of medicinally important compounds. These organ cultures are relatively more stable. There are a number of medicinal plants whose shoot cultures have been studied for metabolites and are valuable sources of medicinal compounds [29, 30]. There are also leaf cultivation for extracting the secondary compound and testing for its activity such as in 2005, Iwase. A., et al, repoted that a novel system was developed for producing Ajmalicine and Serpentine as useful secondary metabolites by direct culture of leaves in *Catharanthus roseus*. *C roseus* was used as a model medicinal plant to produce terpenoid indole alkaloids (TIAs) by suspension culture of the leaves in the phytohormone-free MS liquid medium [34]. There are also attemps to create secondary metabolites for pharmaceutical aspect by using plant leaf tissue culture besides shoots and roots culture. Recently Sukrong, S., et al. has studied on plant cell culture and plant callus induction and growth such as *Punica granatum*, *Phaseolus vulgaris* L., *Morus alba* L. and *T. laurifolia* especially *T. laurifolia* (TL) which is a widely useful Thai traditional plant but has not been reported about its callus induction and creation of secondary metabolite from callus.

In this study, we focused on a callus induction from *T. laurifolia* leaves and extracted a secondary metabolite from the callus culture. The extraction from *T. laurifolia* callus was measured for second metabolites to use as active substance using rosmarinic acid as a chemical marker.

3. Rosmarinic acid

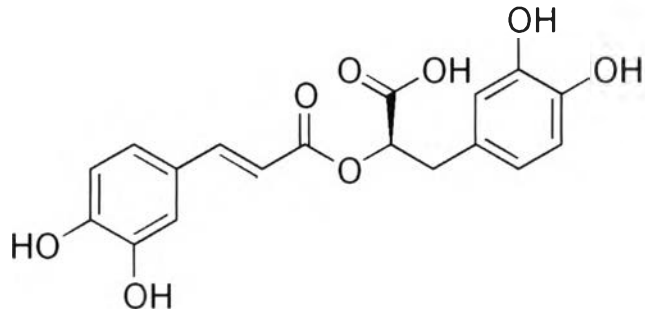


Figure 9 Structure of rosmarinic acid

Rosmarinic acid (RA) is a polyphenol ester found in a variety of herbal plants especially in the Lamiaceae group such as rosemary, sage, spanish sage, oregano. It has a variety of bioactivities especially antioxidant and anti-inflammatory properties. It also can breaks-up amyloid-beta conglomerates of Alzheimer's Disease in laboratory studies, anti-viral with effects against Herpes Simplex [35-38]. In addition, there was also reported an antioxidant activity of rosmarinic acid from *T. laurifolia* showed the significant DPPH-scavenging capacity with an EC-value of 2.71 µg/ml [15].

The structure of rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllacticacid.The IUPAC name is (2"R")-2-[[(2"E")-3-(3,4 dihydroxyphenyl)-1-oxo-2-propenyl]] oxy] -3-(3,4-dihydroxyphenyl) propanoic acid. Molecular formula is $C_{18}H_{16}O_8$ and molecular weight is 360.31 g/mol. It is soluble in organic solvent such as ethanol but slightly soluble in water (MSDS for rosmarinic acid). Recently, the antioxidant-guided isolation of rosmarinic acid from *T. laurifolia* was reported to be used as a bioactive marker for standardization. *T. laurifolia* leaves were collected and extracted with 95% ethanol then analyzed by using TLC and HPLC to isolate and standardize rosmarinic acid respectively. The HPLC condition was developec and performed as using C_{18} column and the mobile phase was isocratic water-methanol-acetic acid (65:35:0.1). The flow rate was 1 ml/min and monitored the rosmarinic acid peak with UV at 320 nm [15]. Due to an unstable to heat and oxidation of rosmarinic acid, it is suitable as a model substance for most of herbal extracts to study their formulations and encapsulation processes and its antioxidant activity would be useful in biomedical aspect such as wound healing that will be described more in wound dressing part.

4. Durian fruit hulls polysaccharide

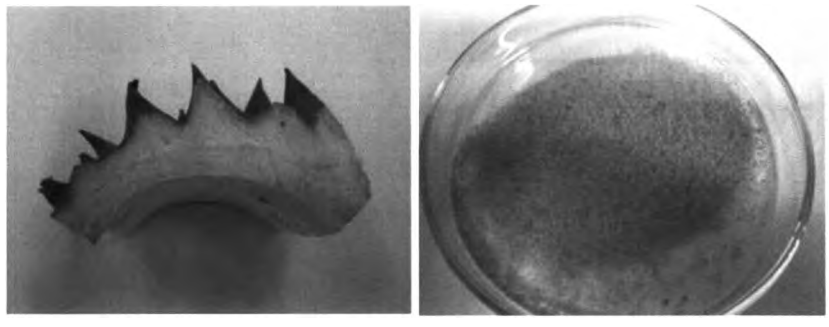


Figure 10 Durian fruit hulls for extraction (left), polysaccharide gel extracted from durian fruit hulls (DG) (right)

Polysaccharide gel from durian fruit hulls (DG), a water soluble polysaccharide isolated from *Durio zibethinus* by hot water extraction followed by ethanol precipitation as the step in figure 11, has a pectic polysaccharide structure, as a principal component. A high amount of $\alpha(1\longrightarrow4)$ linked galacturonic acid confirms the presence of pectic polysaccharide. Pectin is a complex branched heteropolysaccharides primilarily containing $\alpha(1\longrightarrow4)$ polygalacturonic acid backbone. Rhamnose, galactose and arabinose residues were encompassed in the polygalacturonic acids of the pectin. The pectins from *Durio zibethinus* contain arabinogalactans side chains containing β-$(1\longrightarrow6)$ linkage, any neutral sugar side chains in DG might be cleaved leading to lower amount of neutral sugars in side chains, especially arabinose and galactose, than those present in nature because highly acidic condition was involved in the isolation of DG [4].

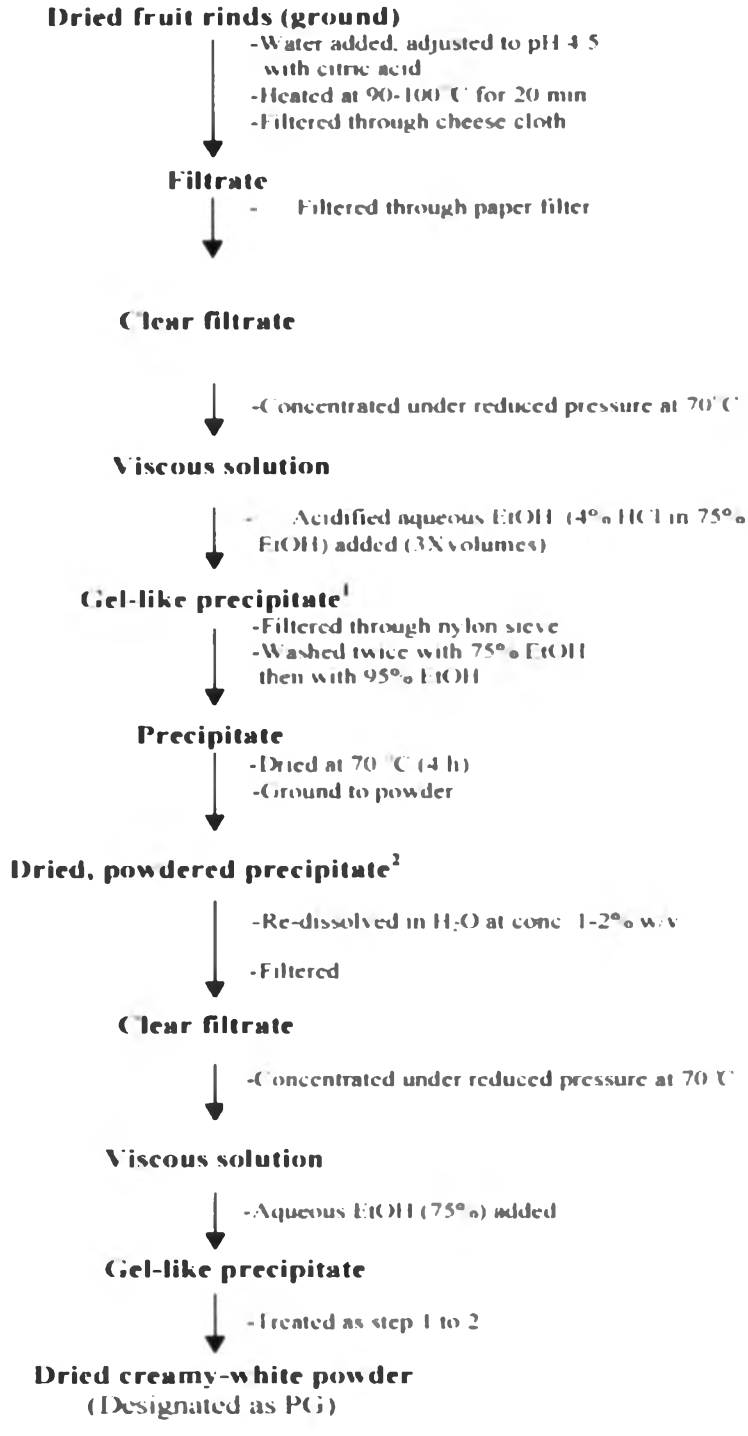
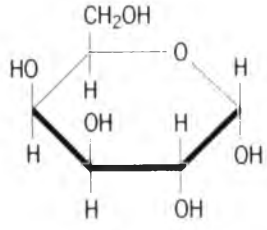
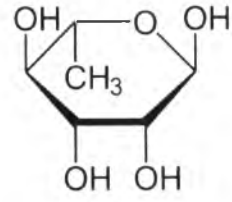


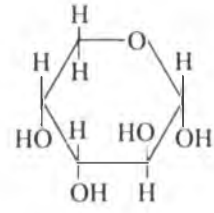
Figure 11 The scheme of extraction DG from durian fruit hulls [4].



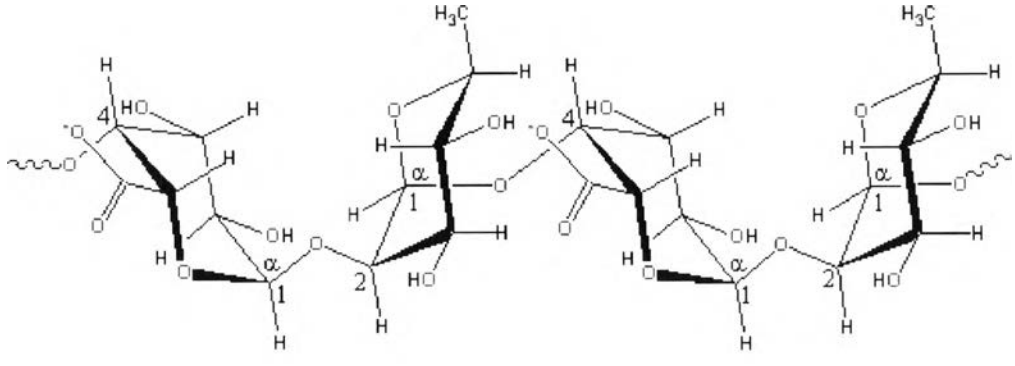
Galactose



Rhamnose



Arabinose



Pectin

Figure 12 Structure of galactose, rhamnose, arabinose residues and pectin

DG was evaluated its antimicrobial property against bacteria and yeasts by agar diffusion test. Several strains of bacteria inhibited by DG were *Bacillus subtilis*, *Micrococcus luteus, Staphylococcus epidermidis, Lactobacillus pentosus, Escherichia coli, Staphylococcus aureus* and *Proteus vulgaris* while 2 strains of yeasts, *Saccharomyces cervisiae* and *Candida albicans*, were not inhibited [7, 39]. The toxicity study and antimicrobial activity were also observed. Toxicity studies showed DG was safe for using high dose or long term of DG in mice and rats [5, 6].

DG can be used to prepare several types of wound dressings such as films, gels and freeze-dried products. DG film and gel wound dressings were examined promotion of wound healing in pig skin [10]. Data showed faster and better wound healing process than traditional treatment: povidone iodine. DG film and freeze-dried patch wound dressings were investigated in pig and dog skin wounds. DG products also gave more advantages for healing process [9, 10]. DG was also used to produce wound dressing patch and compared with commercial products. In 2009, there was a report on processing parameters of the hydrogel dressing prepared by physically cross-linked polyvinyl alcohol (PVA) with polysaccharide gel extracted from durian fruit-hulls (DG) using freeze-thaw technique [8]. Joyce. E.J., et al have studied on

cellular viability of the hybrid DG/PVA hydrogel and reported that DG did not impact the viability of human blood-derived monocytes but did decreased adherent cell density and cytoplasmic spreading [40]. This improvement of membrane properties by addition of a polysaccharide gel from durian fruit-hulls extract into polyvinyl alcohol hydrogel and the cellular viability results supports that DG is suitable for further development in biomedical applications [40].

Since DG is a bioactive polymer and developed from unusable agriculture waste to value added by-product for pharmaceutical and healthcare industries, it is an interesting polymer to perform in an encapsulation process in this study.

5. Sodium alginate

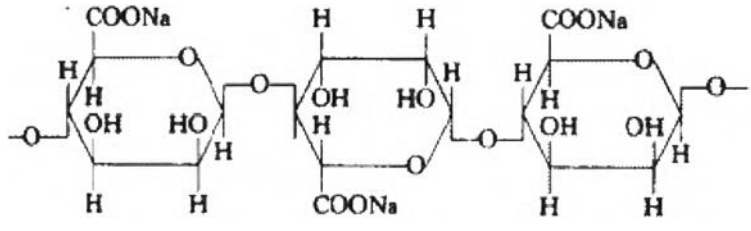


Figure 13 Structure of sodium alginate

Sodium alginate, an anionic polysaccharide, has a wide range used in microencapsulation technique for drug delivery systems due to its biocompatibilty, biodegradability and nontoxic properties. Alginate is distributed widely in the cell walls of brown algae, and forming a viscous gum through water binding. The extraction powder has high capacity of water absorption approximately 200-300 times of its own weight.

Alginic acid is a linear chain copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks. The monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks) or alternating M and G-residues (MG-blocks). Due to alginate's biocompatibility and simple gelation with divalent cations such as Ca^{2+} which the crosslinking is called egg-box model for hardening the particle structure as figure 14-15, it is widely used for cell immobilization and microencapsulation as release controlled and delivery system [11].

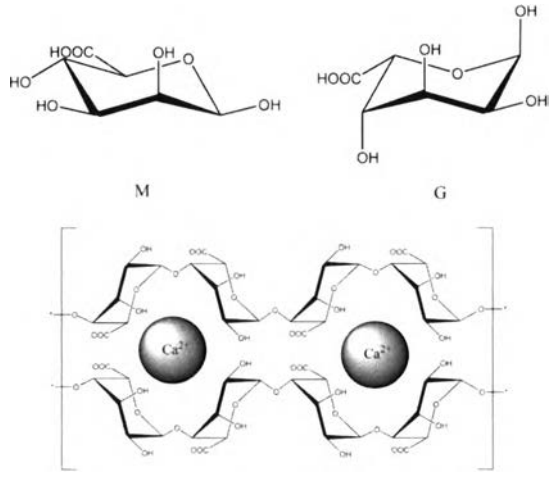


Figure 14 Calcium crosslinked of sodium alginate of MG-blocks

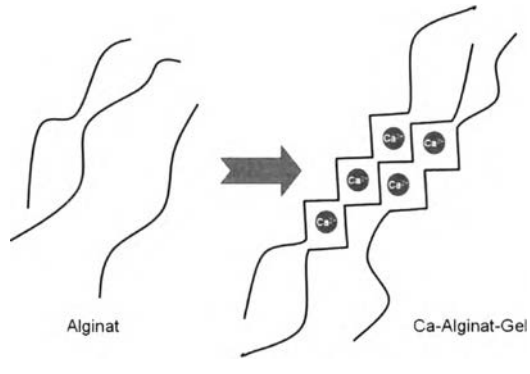


Figure 15 Egg-box model of of sodium alginate calcium crosslinking

In 1999, Liu. P. reported a combining of polymer alginate, pectin, and polylysine which prepared for controlled release formulation. Alginate and pectin served as a core polymer and with polylysine to strengthen these particles. Using of pectin has advantage in forming a more robust particulate system which has more acidic pH resistant and modulated a release profiles of the model encapsulated drugs in the alkaline pH [41]. Also in 2004, H. Madziva., et al. reported that a blending of alginate and pectin polymer, the microparticle has increased in folic acid encapsulation efficiency and showed higher folic acid retention after freeze drying and storage with reducing of leakage from the capsules compared to those with alginate alone [42]. A novel preparation techniques for alginate–poloxamer microparticles controlling protein release on mucosal surfaces with a size range suitable for pulmonary administration was also developed and reported in 2011. In that study, bovine serum albumin (BSA)-loaded microparticles were prepared by

spray drying aqueous polymer drug solutions, followed by cross-linking the particles in aqueous or ethanolic $CaCl_2$ or aqueous $ZnSO_4$ solutions. Protein release of the cross-linked microparticles from ethanolic cross-linked solution was much faster than that of aqueous solutions. The cross-linked microparticles of alginate and poloxamer can be produced in a size range which appropriate for deep lung delivery and with controlled protein release kinetics [43].

The cross-linked microparticles from polymers having structure similar to alginate and pectin offered an interesting potential for further develop to control delivery and biomedical applications.

6. Wound dressing

Wound healing is a complex and dynamic process of replacing devitalized and missing cellular structures and tissue layers. The human adult wound healing process can be divided into 3 or 4 distinct phases. Earlier authors referred to 3 phases: inflammatory, fibroblastic, and maturation, which had been denoted in earlier versions as inflammatory, proliferative, and remodeling and this is maintained by some authors. In the 4-phases concept, there are the hemostasis phase, the inflammatory phase, the proliferative phase, and the remodeling phase. In the 3-phases approach, the hemostasis phase is contained within the inflammatory phase [44].

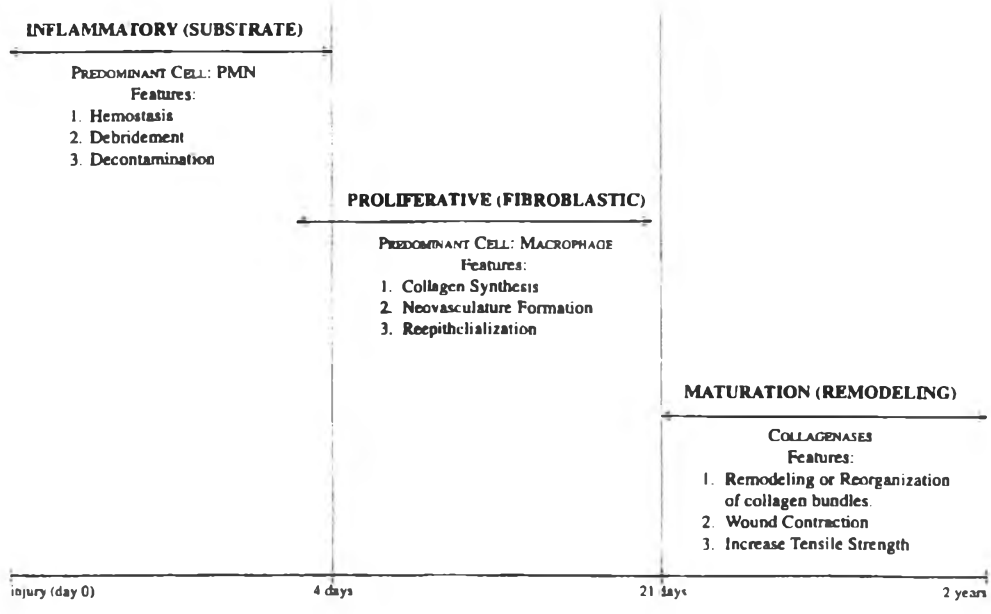


Figure 16 Timeline of wound healing phases [44]

The inflammatory phase is the body's natural response to injury. After initial wounding, the blood vessels in the wound bed contract and a clot is formed. Once haemostasis has been achieved, blood vessels then dilate to allow essential cells; antibodies, white blood cells, growth factors, enzymes and nutrients to reach the wounded area. This leads to a rise in exudate levels so the surrounding skin needs to be monitored for signs of maceration. The predominant cells at work here are the phagocytic cells; neutrophils and macrophages mounting a host response. During proliferation, the wound is rebuilt with new granulation tissue which is comprised of collagen and extracellular matrix and into which a new network of blood vessels develop, a process known as angiogenesis. Healthy granulation tissue is dependent upon the fibroblast receiving sufficient levels of oxygen and nutrients supplied by the blood vessels. Healthy granulation tissue is granular and uneven in texture. Epithelial cells finally resurface the wound, a process known as epithelialisation. Maturation is the final phase and occurs once the wound has closed. This phase involves remodeling of collagen from type III to type I. Cellular activity reduces and the number of blood vessels in the wounded area regress and decrease [44].

Oxidative stress plays some role in wound healing as in figure 17. Reactive oxygen species (ROS) are produced by all cells during the course of normal metabolic processes, e.g. in the respiratory chain. Particular large amounts are produced in wounded and inflamed tissue. This phenomenon has been described as the "respiratory burst". If the detoxification of ROS is insufficient or if ROS are

produced in excessive amounts, oxidative stress occurs, resulting in severe cell damage, premature aging or even neoplastic transformation. Several low molecular weight antioxidants have been suggested to regulate the redox environment in healing skin wounds. These include endogenous molecules, such as glutathione, ubiquinones, uric acid, and lipoic acid, but also compounds present in food, such as vitamins E and C (ascorbic acid), carotinoids, and phenolic compounds. The functional importance of these antioxidants in the wound repair process is suggested by their depletion in healing skin wounds [16].

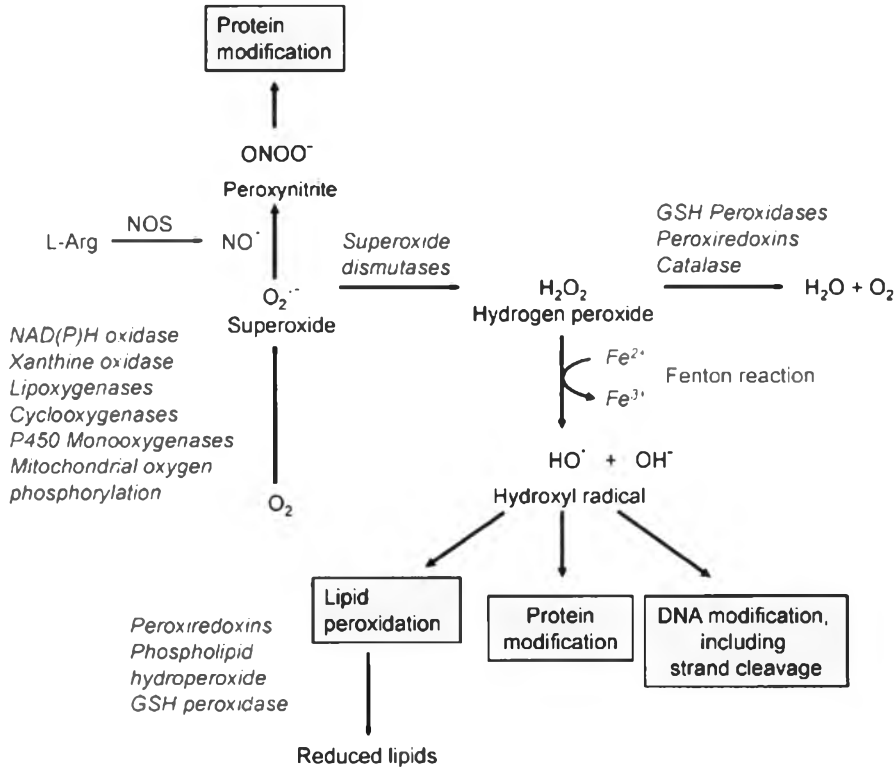


Figure 17 Pathway of reactive oxygen species that produced in inflamed tissues [16]

Wound dressing is a material used by a person for application to a wound to promote healing or prevent further harm. They are frequently used in first aid and nursing. The dressings will be selected depend on type, position, and severity of the wound, although all purposes are focused towards promoting wound healing, recovery and preventing further harm to the wound. Key purposes of wound dressings are healing the wound, absorbing exudate, protecting from infection and promoting healing [45].

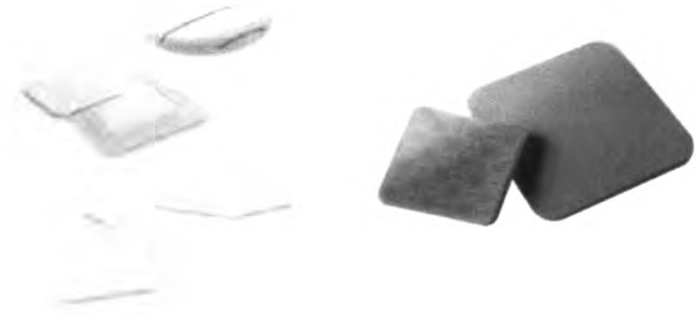


Figure 18 Dressing patches used for wound dressing

There are many types of wound dressing depend on materials such as emollient dressing, film dressing, hydrocolloids, hydrogels, calcium alginate dressings, hydro-fiber dressing, foam dressing, and freeze-dried dressing patch. The polymeric dressings employed for controlled drug delivery to wounds include hydrogels such as poly (lactide-co-glycolide), poly (vinyl pyrolidone), poly (vinyl alconol) and poly (hydroxylalkylmethacrylates) polyurethane-foam. Other polymeric dressings reported for drug delivery system to wounds compose of novel formulations prepared from polymeric biomaterials such as hyaluronic acid, collagen and chitosan. The biopolymers are more effective as a wound-healing accelerator comparing to the synthetic polymers. The wound treated with biopolymers and biomaterials shows accelerated healing. Biopolymers structural arrangement is similar to the normal skin. Hence, the biopolymers are considered to be one of ideal materials because of their biocompatibility, biodegradability, and wound healing property as well as easy application [45, 46].

Table 3 Class of wound dressing, indications and commercial products [44]

Class	Indication	Example
Transparent Film	Dry wounds—prevention desiccation, encourage moist environment	Bioclusive™ (Johnson & Johnson) POLYSKIN II™ (Kendall)
Hydrogels	Lightly exudative—nonadherent, encourage moist environment	NU-GEL™ (Johnson & Johnson)
Impregnated gauzes	Nonadherent (open mesh)—moderate to heavily exudative, prevent desiccation	Adaptic™ (Johnson & Johnson) Aquaphor™ (Beiersdorf)
	Nonadherent (fine mesh)—dry to lightly exudative, encourage moist environment	Petrolatum gauze U.S.P (Sparta) Xeroform™ (Sparta)
	Contaminated/superficially infected wounds	Betadine™ Antiseptic Gauze (Purdue Frederick)
Nonadherent	Lightly exudative—absorbent, prevent maceration	Release™ (Johnson & Johnson) Telfa™ (Kendall)
Wet dressings	Dry, crusted, desiccated wounds	Sterile water wet dressing (Sparta) Isotonic saline wet dressing (Sparta)
	Draining, contaminated wounds	Hypertonic Wet Dressing (Sparta)
	Clean, dry wound—nonadherent	Owens Dressing (American Cyanamid)
Hydrocolloids	Lightly exudative wounds—provide occlusion and promotes moist environment	DuoDERM™ (Convatec)
	Good when autolytic debridement is desired	
Calcium alginate	Moderately to heavily exudative wounds—absorbent, prevent maceration	Sorbsan™ (Dow B. Hickam)
Exudate absorbers	Moderate to heavily exudative wounds—absorbent, enhance autolytic debridement	Debrisan™ (Johnson & Johnson)
Composite dressings	Various uses depending on composition of dressing	Mitraflex™ (Calgon Vestal)
Silicone gel	Hypertrophic scars and keloids—decrease size and reduce pain/itching	SILASTIC™ gel sheeting (Smith & Nephew United)
	Prophylactic treatment for known scar/keloid formers	
Wound sprays	Dry or granulating wounds—encourage granulation, maintain moist environment	Dermagran™ moisturizing spray (Derma Sciences)

Engineered scaffold dressings either from natural or synthetic sources are potentially useful for the delivery of additional bioactive materials such as growth factors and bioactive substances to a wound. The active ingredients used in wound management have evolved alongside the pharmaceutical agents and dressings which used to be delivery system. The incorporated drugs play an active role in the wound healing process either directly or indirectly as cleansing or debriding agents for removing necrotic tissue, antimicrobials which prevent or treat infection or growth factors to aid tissue regeneration. Drug release in the wound area can be controlled with particulate system, and this increases the speed of wound healing. The micro-/nanoparticulate systems has been used in wound and burn treatment such as poly (ethylene-co-vinyl alcohol), nanofiber silver nanoparticles, collagen sponges and liposomes containing growth factor [46]. Biocompatible and biodegradable polymer scaffolds combined with cells or biological signals or particulate systems are being

investigated as alternatives to traditional options for tissue engineering that enhance wound healing and skin regeneration.

In this study, the research was focused on the development of the microencapsulation of an extract obtained from *T. laurifolia* callus. The callus extract composed of rosmarinic acid and its antioxidant activity could be useful in accelerating wound closure. For the biomedical application, the device for wound dressing was developed as DG freeze-dried patch since it has a good absorbability of an exudate on wound area and this freeze-dried patch was incorporated with the particulate system for active substance delivery, improve release and stability.